

REMARKS

The Office Action dated December 19, 2003 states that the instant application was found to be eligible for continued examination under 37 C.F.R. § 1.114, and that Applicants' amendment filed July 25, 2003 was entered.

Claims 1 and 11 are amended to insert an omitted phrase and not in response to any rejection relating to patentability.

The Office Action states that because claims 33-35 were misnumbered, these claims were renumbered by the Examiner as claims 34-36 respectively. Applicants thank the Examiner for pointing out and correcting this inadvertent clerical error. In this context, claim 35 has been amended to correct the dependency of this claim on claim 34.

The Office Action further states that non-elected claim 33 is pending in the instant application. Applicants respectfully disagree with this statement, because claim 33 was cancelled in a Preliminary Amendment filed June 17, 1999 (Express Mail Label No. EL 110243543US).

Accordingly, claims 1-11, 14-27 and 34-36 are pending in the instant application.

Objection

The Office Action states that there is a spelling error on page 93, *i.e.*, "R fren s". Applicants have not been able to locate this spelling error. In particular, the substitute specification filed July 25, 2003, has only 92 pages. The marked-up copy of the specification has the correctly spelled word "References" on page 93. "References" also appears at page 80 of the clean copy of the substitute specification. Applicants therefore request clarification as to the specific location of the spelling error or a copy of the page bearing the error.

Rejections under 35 U.S.C. § 103(a)

(i) The Office Action maintained the rejection of claims 1-11, 14-18, and 34-36, under 35 U.S.C. § 103(a), as being unpatentable over WO 97/35991 in view of Golden *et al.* (*J. Immunol. Meth.*, **206**:163-9, 1997), O'Shea *et al.* (*Science*, **245**:646-8, 1989), Garboczi *et al.* (*J. Immunol.*, **157**:5403-10, 1996), and Schatz (*Biotech.* **11**:1138-43, 1993).

Specifically, the Office Action suggested that WO 97/35991 teaches soluble, recombinant divalent and multivalent (including tetravalent) analogs of heterodimeric proteins such as $\alpha\beta$ TCR that possess enhanced affinity for their target molecules, wherein the $\alpha\beta$ TCRs are associated via Ig linker molecules which may further comprise a toxin and/or may be further linked by association via avidin. Golden *et al.* is relied upon as teaching soluble heterodimeric TCR, comprising α and β chains, each chain comprising a leucine zipper which dimerizes, one with the other. O'Shea *et al.* is relied upon as teaching heterodimer formation through leucine zippers from c-fos and c-jun. Garboczi *et al.* is relied upon as teaching a soluble TCR without the interchain disulfide bond present in native TCRs, and that heterodimerization, refolding and antigenic specificity of the TCR do not require such an interchain disulfide bond, transmembrane segments or glycosylation. Finally, Schatz is relied upon as teaching biotinylation of proteins.

The Office Action states that it would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to have made a recombinant TCR as taught by the combination of WO 97/35991 and Golden *et al.* without the disulfide bond, as taught by Garboczi *et al.* and further modified as taught by Schatz. According to the Office Action one of ordinary skill would have been motivated to combine these references because Garboczi *et al.* teach that the presence of a disulfide bond is not important for heterodimerization and refolding, and further in order to increase the yield of correctly folded soluble TCR molecules.

To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. A prior art reference must be considered in its entirety, *i.e.*, **as a**

whole, including portions that would lead away from the claimed invention. Second, there must be a reasonable expectation of success. Finally, the prior art references must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in applicants' disclosure.

Applicants note that Golden *et al.* and Garboczi *et al.* are critical to the reasoning provided in the Office Action. If either, or both, of these references do not adequately support the interpretation stated in the Office Action, the entire objection is unsustainable. Applicants submit that upon a proper reading of Golden *et al.* and Garboczi *et al.* one of ordinary skill in the art would not find any teaching, motivation, or expectation of success to arrive at Applicants' claimed invention.

The Office Action states that Garboczi *et al.* teaches that the disulfide bond present in native TCRs is not required for heterodimer formation. Respectfully, Applicants firmly disagree with this characterization of the Garboczi *et al.* reference. Specifically, with respect to Figure 4, a SDS-PAGE gel, the Examiner states that:

Garboczi *et al.* teach "As expected, under nonreducing conditions no disulfide-bonded heterodimer was seen" (especially page 5407, column 1 at lines 8-10), indicating that ... with regard to the native gel shift electrophoresis, the noncovalently associated heterodimer was not expected to be observed as a complex, but as separate chains. (Office Action, page 6, first full paragraph).

and that:

It is the Examiner's position that the native gel shift electrophoresis assay under nonreducing conditions was used to assess the composition of the heterodimer, i.e., to confirm that the heterodimer was consisted of both α and β chains and not homodimers of one chain or the other, and to confirm that the TCR was correctly refolded to bind MHC/peptide. The absence of noncovalently associated heterodimer on native gel shift electrophoresis assay under nonreducing conditions is indicative that when the heterodimer is subjected to the nonphysiologic condition of having an electric field applied, the non-covalently associated heterodimer does not migrate as a unit. (Office Action, page 6, first full paragraph).

Applicants respectfully contend that the statement in Garboczi *et al.* that "As expected, under nonreducing conditions *no disulfide-bonded heterodimer* was seen" [emphasis added] addresses the fact that the "short-form" constructs run on the SDS-PAGE in Figure 4 *did not*

form disulfide-bonded heterodimers “as expected” because these constructs do not contain the cysteine residues required to form the disulfide interchain bonds. Therefore, Applicants disagree with the Office Action’s conclusion as to what the reference teaches because:

(i) the quote clearly refers to the *lack* of non-disulfide-bonded heterodimers; and

(ii) the data presented in Figure 4 relates to an SDS-PAGE gel and *not* a native gel as suggested in the Office Action. The SDS in this gel is, of course, expected to denature the TCR α and β chains. Therefore, Figure 4 provides no assistance in answering the question of the non-covalent association of the TCR chains under native conditions, one way or the other.

The Office Action also suggests that the gel filtration chromatography in Garboczi *et al.* would yield a non-covalently-linked heterodimer. Applicants respectfully disagree. Garboczi *et al.* teach:

The refolded noncovalently linked $\alpha\beta$ heterodimer elutes during gel filtration chromatography at a volume expected for a 40-kDa protein when compared with the elution of standard proteins (data not shown). The calculated molecular mass of the heterodimer is 50.2kDa. (top of left column of page 5407).

Applicants would like to point out that Garboczi *et al.* does not give the calibration data for the gel-filtration versus standard proteins. Furthermore, this reference provides no data regarding the elution of an inter-chain cross-linked heterodimer during gel-filtration. Data concerning the latter would have provided the ordinary skilled artisan with a benchmark for the elution of a *true* heterodimer. Without the calibration data, and without a true heterodimer benchmark, it is not possible for one of ordinary skill in the art to interpret the results quoted in Garboczi *et al.* However, it seems that the non-associated chains have molecular weights of up to 27.5kDa (*see*, Figure 4 of Garboczi *et al.*). 40kDa lies midway between 50.2kDa (the calculated molecular mass of the heterodimer) and 27.5kDa (molecular mass of the non-associated chains), so *prima facie* it was not a heterodimer that was being eluted. Actually, it is impossible to conclude from the gel filtration data just what was eluted at 40kDa, but the fact that the molecular mass of the eluted product is intermediate between the weights of the heterodimer and the monomers reasonably supports the view that there was some form of dynamic association and dissociation going on. This view is entirely consistent with the native

PAGE results depicted in Figure 5, which show that no non-covalently-linked TCR heterodimer is formed absent the MHC. The fact that Garboczi *et al.* asserts that the 40kDa fraction is the “refolded noncovalently linked $\alpha\beta$ heterodimer” which elutes at 40kDa ***does not make it so.***

Taking the teaching of Garboczi *et al.* ***as a whole***, it would be readily apparent to one of ordinary skill in the art that the ***only*** evidence in this reference of the formation of a noncovalently linked $\alpha\beta$ heterodimer derives from the observation from Figure 5 that the two TCR chains came together to bind the peptide-MHC complex. Figure 5 evidences that noncovalently associated TCR/MHC/peptide complex can form, but does not provide evidence for the stable association of the chains in the absence of the peptide-MHC complex.

Turning next to Golden *et al.*, Applicants respectfully submit that this reference ***teaches away*** from the present invention. Golden *et al.* describes the production of murine D10 T-cell receptor in *E.coli* as a secreted leucine zipper fusion protein. The heterodimeric TCR of Golden *et al.* (in which the TCR chains are disulfide-linked and have C-terminal leucine zippers) was detected by conformationally sensitive TCR-specific antibodies only under non-reducing conditions. Golden *et al.* teaches:

Under reducing conditions, all three antibodies failed to detect sD10TCR-LZ protein (Fig. 5, lanes 4, 5, 6), implying that recognition by these conformationally sensitive antibodies **is dependent on disulfide bond formation.** (page 169, left column). [emphasis added].

Therefore, since this reference teaches that conformationally sensitive antibodies only recognize the TCR heterodimer when the disulfide bonds are intact, Golden *et al.* distinctly ***teaches away*** from modifying the TCR components as taught by Applicants.

Because there is no motivation to combine the teachings of Golden *et al.* with those of Garboczi *et al.*, and because there is no expectation of success to arrive at Applicants’ claimed invention based on the cited references, Applicants respectfully submit that this rejection has been applied improperly, and request that this rejection be reconsidered and withdrawn.

(ii) Claims 1, 24 and 25 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over WO 97/35991 in view of Golden *et al.*, O'Shea *et al.*, Garboczi *et al.*, Schatz and U.S. Patent No. 5,635,363.

WO 97/35991, Golden *et al.*, O'Shea *et al.*, Garboczi *et al.*, Schatz have been relied upon for the reasons discussed above. U.S. Patent No. 5,635,363 is relied upon to disclose soluble MHC/peptide tetramers which are biotinylated and multimerized with streptavidin or with avidin and which further comprise a light detectable label or an enzyme and which may further be bound to an insoluble support such as a bead, *i.e.*, a "solid structure", for the purpose of assay.

As explained above, at least one element of the two independent claims of the present invention, namely that the "***disulfide bond present in native TCRs between the α and β or γ and δ chains adjacent to the cytoplasmic domain, is absent from the recombinant TCR,***" is simply not taught or suggested by any of the claimed references, either alone or in combination. In particular, careful review of the experiments of Golden *et al.* and Garboczi *et al.* demonstrate that these references ***teach away*** from removal of the disulfide bond. Thus, there is no teaching, suggestion or motivation to arrive at Applicants' claimed invention. Accordingly, Applicants respectfully request that this rejection be reconsidered and withdrawn.

(iii) Claims 1, and 19-24 were rejected under 35 U.S.C. § 103(a) as being unpatentable over WO 97/35991 in view of Golden *et al.*, O'Shea *et al.*, Garboczi *et al.*, and further in view of Ahmad *et al.*

The Office Action relied on WO 97/35991, Golden *et al.*, O'Shea *et al.*, and Garboczi *et al.*, as discussed earlier. Ahmad *et al.* is invoked to teach attachment of a biotinylated targeting antibody to the surface of a liposome containing biotinylated phosphatidylethanolamine by means of an avidin linker and to teach that liposomes containing lipid derivatives of polyethylene glycol have circulation times sufficiently long to allow for effective *in vivo* drug delivery.

As explained above, at least one element of the two independent claims of the present invention, namely that the "***disulfide bond present in native TCRs between the α and β or γ and***

δchains adjacent to the cytoplasmic domain, is absent from the recombinant TCR,” is simply not taught or suggested by any of the claimed references, either alone or in combination. In particular, careful review of the experiments of Golden *et al.* and Garboczi *et al.* demonstrate that these references *teach away* from removal of the disulfide bond. Thus, there is no teaching, suggestion or motivation to arrive at Applicants’ claimed invention. Accordingly, Applicants respectfully request that this rejection be reconsidered and withdrawn.

CONCLUSION

Claims 1-11, 14-27 and 34-36 were pending in the application. Claims 1, 11 and 35 have been amended by the present submission.

Applicants respectfully request reconsideration of the application in light of the amendments and remarks made herein. If the Examiner believes that a telephonic interview would expedite the allowance of the application, the Examiner is invited to contact the undersigned attorney at the number below.

No fees are believed to be due in connection with this matter. However, if any fees are due, the Commissioner is hereby authorized to charge the requisite fees to Deposit Account No. 08-0219.

Respectfully submitted,
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